SUPPLEMENTAL DATA

Arabidopsis Calmodulin-like Protein CML36 is a Calcium (Ca^{2+}) Sensor that Interacts with the Plasma Membrane Ca^{2+}-ATPase Isoform ACA8 and Stimulates its Activity

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Supplementary Figures

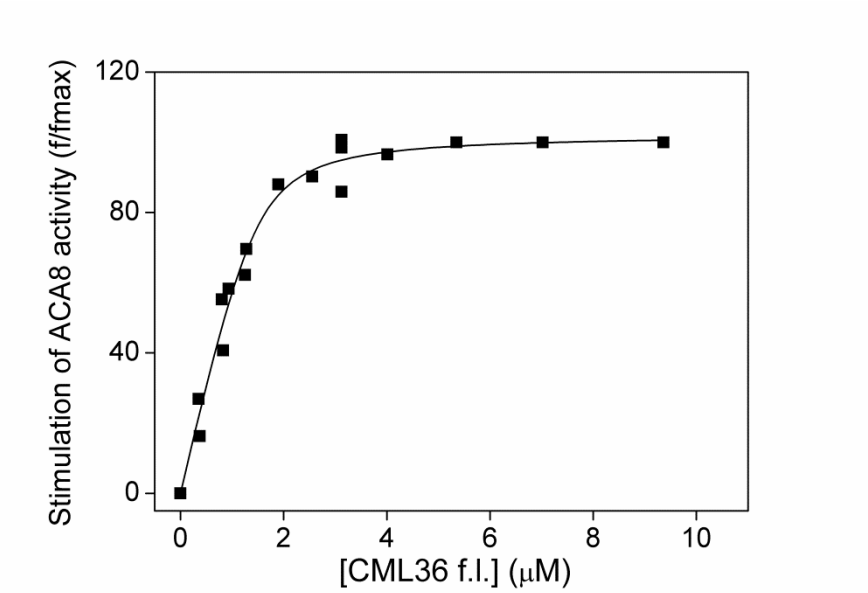

Figure S1. Ca^{2+}-ATPase activity of yeast microsomes overexpressing ACA8 as a function of increasing CML36 full length concentrations.

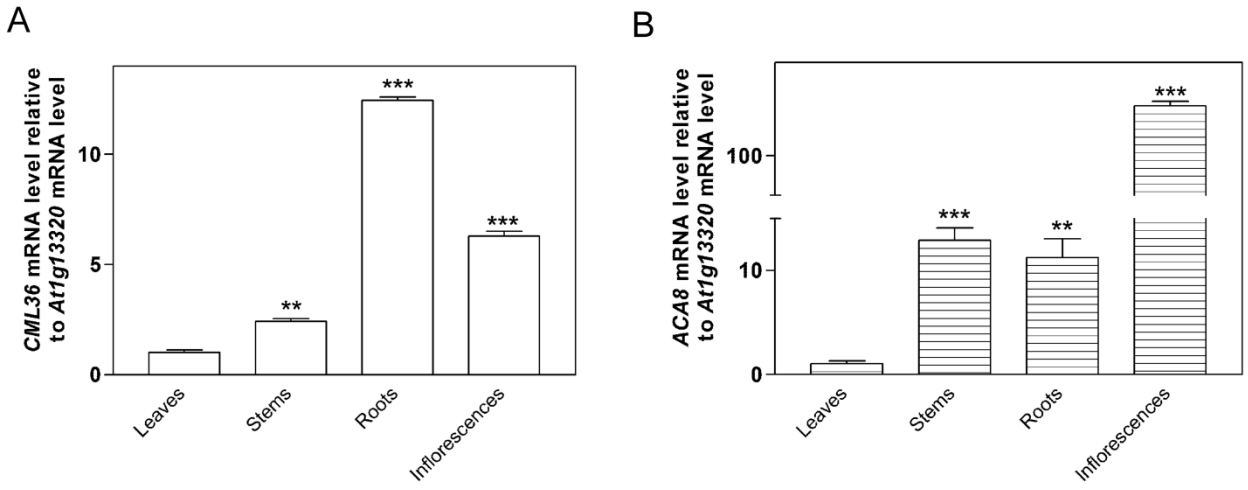

Figure S2. Expression pattern analysis of *CML36* (A) and *ACA8* (B) in various organs of wild-type adult plants assessed by quantitative real-time PCR. The expression levels were normalized using At1g13320 as endogenous control gene and the relative expression ratios were calculated using leaves as calibrator sample. The values reported are means ± SE (n = 3). Student's *t*-test was applied, **P<0.01; ***P<0.001 vs. the level measured in the leaves.

Supplementary Methods

CML19 expression and purification

The complete cDNA of *Arabidopsis thaliana* CML19 (At4g37010) in pUNI51 vector was obtained from Arabidopsis Information Resource (TAIR). The clone contained a premature stop codon, which was corrected using Quick Change Mutagenesis kit (Agilent). To produce recombinant His_6-tagged CML19 the following CML19-specific PCR forward (F) and reverse (R) primers were used: F, (5'- CCATGGGCCATCATCATCATCATTCGGAAGCAGCAC-3'), and R, (5'-GGATCCTTAGCCGTAAGAGGTTCTCTTCATCATCTTC-3'). The F primer carried a *NcoI* restriction site and a 6 His-tag, while the R primer contained a stop codon and a *BamHI* restriction site. The verified cDNA sequence was then sub-cloned into pET15b prokaryotic expression vector for expression in *E.coli* Rosetta cells. Cells were grown in LB medium at 37 °C to a turbidity of 0.6–0.8 at 600 nm. Expression was induced with 0.4 mM IPTG for 18 h at 25 °C. Cells were harvested and resuspended in 5 mM Tris-HCl pH 7.5, 150 mM KCl, 10 mM imidazole and incubated at room temperature for 30 minutes in the presence of 0.1 mg/ml lysozyme, 100 µg/ml DNAase (Sigma), 10 mM $MgCl_2$. The culture was then sonicated on ice. After centrifugation, the supernatant containing the soluble protein was loaded to a Ni-sepharose column which was pre-equilibrated with 5 mM Tris-HCl pH 7.5, 150 mM KCl and 10 mM imidazole buffer. A linear gradient from 10 to 500 mM imidazole was then applied. Under these conditions, CML19 elutes from the column around 150 mM imidazole. The fractions containing CML19 were pooled, extensively dialyzed against 5 mM Tris-HCl at pH 7.5, 150 mM KCl and stored at -80° C until use.